

work, an IL2-Fc fusion protein is used in a “triple therapy” in which it is combined with an IL15-Fc receptor antagonist and rapamycin. Again, these investigators teach that the IL2-Fc fusion protein must have Fc effector functions to be efficacious, and further teach that this IL-2-Fc fusion protein must be combined with two other molecules in order to be efficacious.

[0012] This invention provides for a novel therapeutic agent, an IL2 Selective Agonist – Fc fusion protein with a peptide linker of from 6-30 amino acids. This configuration combines the high cellular selectivity of a IL2 Selective Agonist for Treg cells with a long circulating half-life. In the course of developing this molecule, there were surprising and unexpected findings that revealed structural elements and design features of the protein that are essential for bioactivity, and that led to the discovery of several novel proteins that fulfill the desired therapeutic characteristics.

SUMMARY

[0013] This disclosure provides for a fusion protein between an IL2 $\alpha\beta\gamma$ Selective Agonist protein (IL2 Selective Agonist) and a IgG Fc protein where the IL2 agonist and the Fc protein are separated by a linker of 17 Å to 105 Å and configured so the IL2 agonist is at the N terminus of the molecule and the Fc protein is at the C terminus. The IL2 Selective Agonist moiety provides a therapeutic activity by selectively activating the IL2 $\alpha\beta\gamma$ form of the receptor, thus selectively stimulating Tregs. The Fc moiety provides a prolonged circulating half-life compared to the circulating half-life of IL-2 or an IL2 Selective Agonist protein. The Fc moiety increases circulating half-life by increasing the molecular size of the fusion protein to greater than 60,000 daltons, which is the approximate cutoff for glomerular filtration of macromolecules by the kidney, and by recycling the fusion protein through the Neonatal Fc Receptor (FcRn) protein, the receptor that binds and recycles IgG, thus prolonging its circulating half-life. The Fc moiety will also be deficient in Fc effector functions, such as Complement-Dependent Cytotoxicity (CDC) and Antibody-Dependent Cellular Cytotoxicity (ADCC), enabling the fusion protein to selectively activate Tregs to potentiate Treg function and to expand Treg numbers. The two protein moieties are fused in a manner that maintains robust bioactivity of the IL2 Selective Agonist moiety and enables the Fc moiety to promote a prolonged circulating half-life and thus efficiently potentiate Tregs function and numbers. This potentiation of Tregs will suppress over-exuberant autoimmune or inflammatory responses, and will be of benefit in treating autoimmune and

inflammatory diseases. The proteins of this disclosure may be monomeric or dimeric forming dimers through cysteine residues in the Fc moieties or domains.

[0014] More specifically, this disclosure provides for a fusion protein, comprising: a N-terminal human IL-2 variant protein moiety, and a C-terminal IgG Fc protein moiety, wherein said IL-2 fusion protein has the ability to selectively activate the high affinity IL-2 receptor and thus selectively activate human regulatory T cells. The variants of IL-2 include those with substitutions selected from the group consisting of: N88R, N88G, D20H, Q126L, and Q126F relative to human IL2 protein. SEQ ID NO:1 is variant IL-2/N88R, with the numbering corresponding to wt IL2. In addition, the IL-2 variant protein optionally comprises human IL-2 with the substitution C125S. It is preferred that the proteins of this disclosure are fused wherein both the IL-2 variant protein and the IgG Fc protein have an N-terminus and a C-terminus and said human IL-2 variant protein is fused at its C-terminus to the N-terminus of the IgG Fc protein. It is further disclosed that the activity of the IL-2 variant domain is greatly enhanced when a linker peptide positioned between the IL-2 variant protein and the IgG Fc protein moieties. The IgG Fc protein moiety or domain will optionally be deficient in Fc effector functions or contain one or more amino acid substitutions that reduce the effector functions of the Fc portion of the fusion protein.

[0014a] In one aspect, the present disclosure refers to a fusion protein comprising:

- a. a human IL-2 variant protein domain comprising a substitution selected from the group consisting of: N88R, N88G, D20H, Q126L, and Q126F;
- b. a peptide linker domain; and
- c. an IgG Fc protein domain,

wherein each domain has an amino-terminus (N-terminus) and a carboxy terminus (C-terminus); and wherein the fusion protein is configured so that the C-terminus of the human IL-2 variant protein domain is fused through a peptide bond to the N-terminus of the peptide linker domain, and the N-terminus of the IgG Fc protein domain is fused through a peptide bond to the C-terminus of the peptide linker domain.

[0015] An example of this disclosure is a protein, comprising: a IL-2 variant protein having amino acid substitutions N88R and C125S relative to human IL-2 (SEQ ID NO:1 - N88R), a linker peptide as set forth in SEQ ID NO:15, and a human IgG1 Fc protein having the substitution N297A as set forth in SEQ ID NO:2, wherein said fusion protein has the ability to selectively activate the high affinity IL-2 receptor and thus selectively activate human regulatory T cells. Alternative proteins of this disclosure include: a IL-2 variant protein having amino acid substitutions N88R and C125S relative to human IL-2 (SEQ ID NO:1 – N88R), a linker peptide as set forth in SEQ ID NO:15, and a human IgG2 Fc protein as set forth in SEQ ID NO:3.

[0016] A more specific example of this disclosure is a dimeric protein, comprising two identical chains, where each chain comprises a N-terminal human IL-2 variant protein moiety and a C-terminal IgG Fc protein moiety wherein: the N-terminal human IL-2 variant protein moiety has a N-terminus and a C- terminus varies from the human IL-2 wildtype as in SEQ ID NO:1 by at least one of the substitutions selected from the group consisting of: N88R, N88G, D20H, Q126L, and Q126F, has at least a 90 or 95 or 97% sequence identity to

Sequence ID No. 1; and, has the ability to activate Treg cells by binding to a IL2R $\alpha\beta\gamma$ on those cells; the N-terminal human IL-2 variant protein is joined at its C-terminal to a N-terminus of an amino acid linker of between 6 to 20 or 6 to 30 amino acid residues where said linker also has a C-terminus; and, the C-terminus of the amino acid linker is joined to the N-terminus of IgG Fc protein moiety having 90 or 95 or 97% sequence identity to for example SEQ ID NO:3 (IgG2) or SEQ ID No. 2 (IgG1N297A) and containing cysteine residues; and where the two chains are linked to each other through the cysteine residues that form the interchain disulfide bonds of the IgG Fc protein moiety. The dimers of this disclosure may be further substituted at C125S of the IL-2 moiety. The proteins of this disclosure preferably include amino acid linkers consisting a group of glycine residues, serine residues, and a mix of glycine and serine residues. The linkers may comprise a mix of between 12 and 17 serine and glycine residues preferably with a ratio of glycine residues to serine residues in a range of 3:1-5:1, e.g, a 4:1 ratio.

[0016a] In another aspect, the present disclosure refers to a dimeric protein comprising two identical chains, wherein each chain comprises the fusion protein as disclosed herein.

[0017] This disclosure further provides for the compositions above in a pharmaceutical composition comprising a pharmaceutically acceptable carrier.

[0017a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising the fusion protein as disclosed herein.

[0018] This disclosure further provides for nucleic acids encoding the proteins described herein. The nucleic acids are preferably operably linked to expression cassettes that can be either designed for recombination with a host cell genome or introduced on an independently replicating plasmid or extrachromosomal nucleic acid.

[0019] This disclosure further provides for methods of selectively activating human regulatory T cells in a patient in need thereof, the method comprising administering a pharmaceutical composition comprising the compositions described administered at therapeutically effective doses until human regulatory T cell concentrations reach desired levels.

[0020] A method of measuring the numbers of Treg cells in a human blood sample by contacting human blood cells with the fusion protein of claim 1 at a concentration of between 1 nM and 0.01 nM, and then detecting cells that bind to the protein by flow cytometry.

WHAT IS CLAIMED IS:

1. A fusion protein comprising:

a. a human IL-2 variant protein domain comprising a substitution selected from the group consisting of: N88R, N88G, D20H, Q126L, and Q126F;

b. a peptide linker domain; and

c. an IgG Fc protein domain,

wherein each domain has an amino-terminus (N-terminus) and a carboxy terminus (C-terminus); and wherein the fusion protein is configured so that the C-terminus of the human IL-2 variant protein domain is fused through a peptide bond to the N-terminus of the peptide linker domain, and the N-terminus of the IgG Fc protein domain is fused through a peptide bond to the C-terminus of the peptide linker domain.

2. The fusion protein of claim 1, wherein:

a. the human IL-2 variant protein domain further comprises a substitution selected from the group consisting of: T3A and C125S, relative to the amino acid sequence of SEQ ID NO: 1;

b. the peptide linker domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19; and

c. the IgG Fc protein domain comprises an amino acid sequence selected from the group consisting of the human IgG1 Fc variant of SEQ ID NO: 2, the human IgG2 Fc of SEQ ID NO: 3, and the human IgG4 Fc protein of SEQ ID NO: 24.

3. The fusion protein of claim 1, wherein the human IL-2 variant protein domain comprises the amino acid sequence of SEQ ID NO: 1.

4. The fusion protein of claim 1, wherein the IgG Fc protein domain comprises an IgG1 Fc protein comprising an N297A mutation relative to the amino acid sequence of SEQ ID NO: 2.

5. The fusion protein of claim 1, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 9.

6. A dimeric protein comprising two identical chains, wherein each chain comprises the fusion protein of claim 1.

7. The dimeric protein of claim 6, wherein:

i) the human IL-2 variant protein domain further comprises at least one substitution selected from the group consisting of: T3A and C125S relative to the amino acid sequence of SEQ ID NO: 1;

ii) the IgG Fc protein domain

a. comprises an amino acid sequence selected from the group consisting of the human IgG1 Fc variant of SEQ ID NO: 2, the human IgG2 Fc of SEQ ID NO: 3, and the human IgG4 Fc protein of SEQ ID NO: 24; and

b. comprises cysteine residues, and

iii) the two identical chains are linked to each other through the cysteine residues of the IgG Fc protein domain.

8. The dimeric protein of claim 6, wherein each chain is SEQ ID NO: 9.

9. A pharmaceutical composition comprising the fusion protein of claim 1.

10. The pharmaceutical composition of claim 9, wherein:

a. the human IL-2 variant protein domain further comprises a substitution selected from the group consisting of: T3A and C125S, relative to the amino acid sequence of SEQ ID NO: 1;

b. the peptide linker domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18 and SEQ ID NO: 19; and

c. the IgG Fc protein domain comprises an amino acid sequence selected from the group consisting of the human IgG1 Fc variant of SEQ ID NO: 2, the human IgG2 Fc of SEQ ID NO: 3, and the human IgG4 Fc protein of SEQ ID NO: 24.

11. The pharmaceutical composition of claim 9, wherein the human IL-2 variant protein domain comprises the amino acid sequence of SEQ ID NO: 1.

12. The pharmaceutical composition of claim 9, wherein the IgG Fc protein domain comprises an IgG1 immunoglobulin Fc protein comprising an N297A mutation relative to the amino acid sequence of SEQ ID NO: 2.

13. The pharmaceutical composition of claim 9, wherein the fusion protein comprises the amino acid sequence of SEQ ID NO: 9.